PROSPECTIVE STUDY OF PLATELET DERIVED GROWTH FACTOR IN WOUND HEALING OF DIABETIC FOOT ULCERS IN INDIAN POPULATION

Dissertation submitted to
THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
In partial fulfillment of the degree of
M.S. GENERAL SURGERY

Branch- I
PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH
DEPARTMENT OF GENERAL SURGERY
APRIL 2013

CERTIFICATE

This is to certify that DR.G.KANNIGA PRASANTH, postgraduate student (2010-2013) in the department of General Surgery, PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, Coimbatore, has done this dissertation titled "A PROSPECTIVE STUDY OF PLATELET DERIVED GROWTH FACTOR IN WOUND HEALING OF DIABETIC FOOT ULCERS IN INDIAN POPULATION" under the direct guidance and supervision of guide DR.M.G.MAHENDRAN in partial fulfillment of the regulations laid down by The Tamilnadu Dr.M.G.R. Medical University, Chennai, for M.S., Branch – I General Surgery degree examination.

Prof.DR.M.G.MAHENDRAN M.S
Chief Unit 4
Dept. of General Surgery
PSG IMSR

Prof.DR.S.PREMKUMAR M.S
Professor & Head,
Dept. of General Surgery
PSG IMSR

Prof.DR.S.RAMALINGAM M.D
Principal,
PSG Institute of Medical Sciences & Research,
Coimbatore - 641004

DECLARATION

I, Dr. D. G.KANNIGA PRASANTH, solemnly declare that this dissertation "A PROSPECTIVE STUDY OF PLATELET DERIVED GROWTH FACTOR IN WOUND HEALING OF DIABETIC FOOT ULCERS IN INDIAN POPULATION" is a bonafide record of work done by me in the Department of General Surgery, PSG institute of Medical Sciences & Research, Coimbatore, under the guidance of DR. M.G.MAHENDRAN, Professor of Surgery.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of MS Degree (General Surgery) Branch-I, Examination to be held in April 2013.

Place: Coimbatore

Date: 10th December, 2012

(G.KANNIGA PRASANTH)

ACKNOWLEDGEMENT

I wish to thank our Principal for having permitted me to conduct this study in this hospital.

I am ever so grateful to the HOD and the entire faculty of the Department of Surgery for the kind guidance, valuable advice, supervision and encouragement while doing this study.My thanks are also to my colleagues for the considerable help extended to me.I wish to place on record my gratitude to all the patients who have been an integral part of this study. I am indebted to them for their willing participation.

(G.KANNIGA PRASANTH)

ABBREVIATIONS

Hb	:	Haemoglobin
Vs	:	Versus
PR	:	Pulse Rate
BP	:	Blood Pressure
DM	:	Diabetes Mellitus
GF	:	Growth Factor
CBC	:	Complete blood count
PVD	:	Peripheral vascular disease
UKB	:	Urine ketone bodies
DBP	:	Diastolic BP
SBP	:	Systolic BP
FBS	:	Fasting blood sugar
PDGF	:	Platelet derived growth factor
rh-PDGF	:	Recombinant human Platelet derived growth factor
US-FDA	:	United States Food and Drugs Administration

TABLE OF CONTENTS

INDEX	CONTENTS	**PAGE No.**
01	Introduction	01
02	Aim of the Study	03
03	Review of Literature	04
04	Materials and Methods	50
05	Observations and Results	55
06	Discussion	64
07	Conclusion	67
08	Bibliography	68
	Annexure – III : Proforma	74
	Annexure – IV : Master Chart	79

INTRODUCTION

DM is a state of chronic hyperglycaemia producing complications like neuropathy,nephropathy and retinopathy.It is also a strong co factor in causing atherosclerotic disease and dyslipidemia.

The microvascular and macrovascular complication prevalence is 46% and 64% respectively. Diabetes is the commonest cause of non traumatic lower extremity amputations. It has been reported that annually, about 1 to 4 percent of those with diabetes develop a foot ulcer.

Invariably the diabetic foot ulcers are chronic ulcers that are resistant to heal because of multidrug resistant organism growth and microvascular complications.[1,2]

Recent advances in concept of wound healing and the factors and cell types involved in wound healing have opened a pathway for curing chronic ulcers.

PDGF is one among the growth factors important in angiogenesis and regeneration that is used in treating chronic ulcers. PDGF are derived from platelets which contain alpha and beta granules. The rh-PDGF is produced by recombinant DNA process by inserting the human gene for the B chain of the Growth Factor in the yeast saccharomyces cevisiae.[3]

AIM OF THE STUDY

1. To evaluate the efficacy of PDGF over saline dressing in healing of diabetic ulcers of the foot.

2. To compare and analyze the distribution of Diabetic ulcers of the Foot with age, sex, location of the ulcer(plantar or dorsum).

REVIEW OF LITERATURE

HISTORICAL

The first Mention of diabetes dates back in Egypt in 1500BC,where Hesy-Ra mentioned Diabetes and ways to prevent “passing of too much urine”. Later in Greek Aretaeus of Cappodocia gave the first medical description of diabetes, referred it to ‘the melting down of flesh and limbs into urine. The terminology “diabetes” refers to siphon and “mellitus” as honey.

Grant Banting and Herbert Best proved that diabetes in dogs is reversible by providing them a extract from the pancreatic islets of Langerhans from healthy dogs thereby confirming endocrine role of pancreas in 1921.The World Diabetes Day is observed every November 14 in view of Banting`s birthday.[4]

In 1500 Bc papyrus entails the use of honey in local wound application for healing as antiseptic,lint was used as a wound contraction agent and animal grease as a mechanical sealant.[5]

Maibach conducted studies on wound healing using saline dressings in 1960,and recommended superior wound healing as compared to other dressing techniques.[6]

The understanding of concepts of growth factors in wound healing over the decade has brought new field of interest in their usage in chronic ulcers. Various studies conducted in vitro proved their efficacy in cell proliferation and angiogenesis and wound contraction.

Recombinant technology made it possible to acquire adequate quantity of growth factors for human trials. Many such trials of growth factor use in chronic diabetic ulcers were conducted. PDGF is now approved for topical treatment of diabetic neuropathic ulcers by US-FDA.[3]

HEALING,REGENERATION & REPAIR

Healing

"Body replacement of destroyed tissues by the living tissue" or "Integrated series of cellular & biochemical events which restores the functional integrity & regains the strength of injured tissue".[7]

Regeneration

"It is a process of replacement of lost tissue by an identical type of fresh tissue". There is proliferation of surrounding undamaged specialized cells, Seen in- [1] Epidermis [2] Endothelium [3] Liver cells [4] Mucous membrane.[8]

Repair

It is the replacement of tissues by granulation maturing into a scar". e.g. muscle & nervous tissue.[9]

HEALING:

Definition:

"Body replacement of destroyed tissues by the living tissue" or "Integrated series of cellular & biochemical events which restores the functional integrity & regains the strength of injured tissue"

Phases of Healing:

Wound healing & repair are complex processes that involves dynamic series of events.

[1] Coagulation

[2] Inflammation

[3] Fibroplasia, Angiogenesis, Proliferation & Granulation tissue formation.

[4] Epithelization

[5] Collagen Synthesis

[6] Wound contraction / Tissue Remodeling / Scar Maturation.[9]

COAGULATION,INFLAMMATION :

- Inflammation is the first process of wound healing and essentially consists of coagulation and neutrophil aggregation.
- Helps in preventing blood loss, covering wound surface, & holding the wound edges together & thus contributing to the healing process.
- Knighton et al (1982) & Ross (1980) have shown equivocally that fibrin & platelets play an important role in initiating the wound healing.[10]

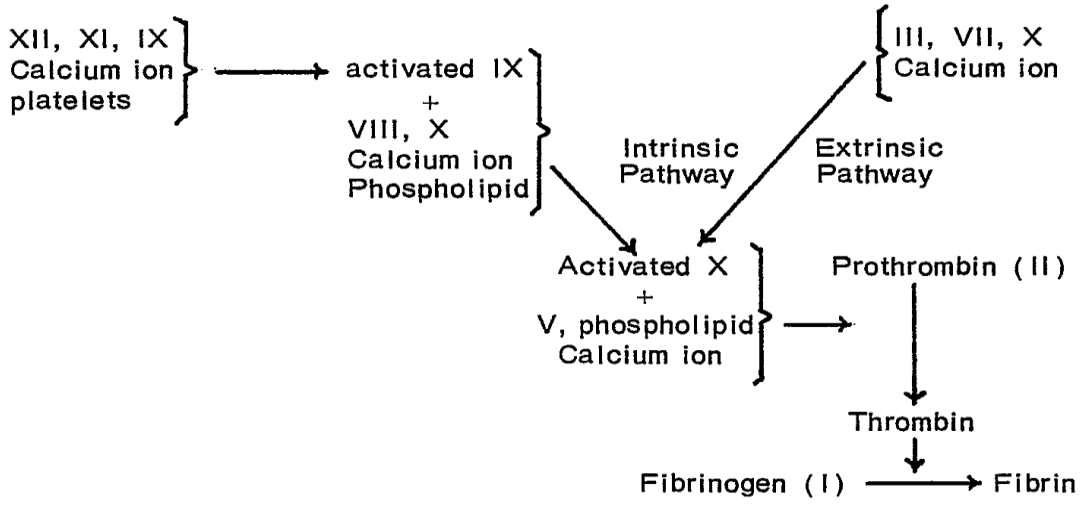


DIAGRAM A

GRANULATION PHASE OF WOUND HEALING :

Phases of wound healing under this phase are : Fibroplasia, Angiogenesis, Proliferation.

A granulation tissue is a highly vascular tissue, containing largely of

1. Fibroblasts [Proliferating fibroblasts + Products of Fibroblasts]
2. Endothelial cells lining capillaries of newly sprouting blood vessels
3. Macrophages
4. Pleuripotent Pericytes

Above all are embedded in a matrix consisting

1. Fibronectin
2. Proteoglycans rich in Hyaluronic acid & collagen [This collagen is at first mainly of Type-III, changing later to Type I].

FUNCTIONS OF GRANULATION TISSUE :-

1. Supports the growing & migrating epithelial cells –The connective tissue matrix of granulation tissue forms nutritive substrate, over which regenerating epidermis can migrate & is gradually replaced by scar tissue.

2. Fill the gap of the wound.

Factors which play important role in Granulation tissue formation:[11]

1. Chemotactic factors
2. Growth Factors
3. Structural molecules
4. Proteases

ANGIOGENESIS[12,13]

Vital part of proliferative phase of wound healing & repair. It is seen in

- Embryonic development phase
- During repair process (throughout life span of an organism)
- Under certain pathological conditions

Without Angiogenesis, invasion of the wound bed by macrophages & fibroblasts would cease due to lack of oxygen & nutrients.

In the initial stages**,** these vessels lack the basement membrane & have loose cellular junction & are fragile in nature. Due to this, on slightest touch, the vessels bleed profusely which is a characteristic feature of newly formed capillaries. The leakage facilitates the movement of cells & macromolecules into wound site.

Step-I : Angiogenesis Initiation

There is disruption of tunica adventitia of blood vessels by proteolysis allowing sprouts of capillary vessels which help in cell migration into the wound site. Angiogenic factors acts on capillary endothelial cells, which releases collagenase. This enzyme degrades the collagen of basement membrane.

Step-II:Angiogenesis Amplification

Fragmentation of the collagen of basement membrane, permits the migration of endothelial cells into the peri-vascular spaces.

Step-III: Vascular Proliferation

Endothelial cells migrate into the peri-vascular spaces where they form buds, which are added by the proliferation of cells within & near parent vessel.

Step-IV:Vascular Stabilization and Angiogenic Suppression

Maturation of endothelial cells & organization into capillary loops

- Functional Capillary Loops: During dermal repair, these buds grow rapidly towards the free surface, where they branch at their tips & unite to form functional capillary loops.

- Superficial Capillary Plexus : On these loops, new buds develop, so that, a superficial capillary plexus rapidly forms in the granulation tissue.
- Canalization : Proliferation & branching of cords of endothelial cells later become canalized to form growing capillary buds of healing wound.
- Fusion : Capillaries originating from opposite sides of the wound fuse & establish a complete circulation with in the wound.

REMODELLING OF THE VASCULATURE:

There is constant remodeling of the vasculature, which involves obliteration of many of the capillaries.

As each capillary loop becomes functional, it brings nutrients & oxygen to nearby cells, enabling the fibroblasts to secrete materials for the matrix, through which macrophages & other cells can migrate further.

As the scar maturation proceeds, capillaries gradually regress & the red vascular rich wound tissue transforms into a white, relatively avascular cell poor scar.

The above proliferative & migratory processes are repeated sequentially, until wound bed is filled with granulation tissue.

MACROPHAGIA [14,15]

- It is a point at which protecting & clearing functions of inflammatory response are linked to starting of reparatory process

Macrophagia is defined as :

[1] Migration of Monocytes [from blood] to tissue injury site

[2] Conversion of monocyte to Macrophage after migration to tissue injury site. These are key cells in dermal repair

- Wound macrophages, which appear subsequent to the cells, play pivotal role in healing by liberating various factors.

Macrophages & angiogenesis[16]

It appears that macrophages promote angiogenesis by liberating Endothelial Growth Factor (EGF).

Macrophages & Collagenase Enzymes : Role of Collagenase

Phase of wound healing	Sources of collagenase	Role of collagenase
In early part of wound healing	Neutrophils	Collagen of wound debris is broken down by collagenase & converted to breakdown products of collagen, which is then cleared by phagocytes, so, they assist in tissue debridement
In later part of wound healing	Macrophages	This enzyme controls the amount of new collagen deposition.

Macrophages & Collagen:

Macrophages secrete lactate which stimulates collagen synthesis by fibroblasts.

Migration of Fibroblasts – Mechanism[17,18,19]

Phases of inflammation	Chemical which acts as chemotactic agent for fibroblasts in their migration :
Initial	By Fibrin – Fibronectin – Collagen Scaffold of wound
Later	By Soluble chemical factor from macrophages & Collagen peptide

Collagenation Mechanism[20] **:**

Tissue replacement by fibrosis and scar formation. The main component of scar tissue & newly laid connective tissue is collagen. It is suggested that , the repaired tissue may contain ≥50% collagen material, & hence this aspect of healing is referred to as collagenation.

Defects in collagenation can give rise to:

1)Poor Healing.

2)Contractures

3)Hypertrophic Scar

Therefore an understanding of underlying bio-chemical,synthetic and remodeling processes leading to the formation of collagen is essential for the rational approach to wound repair. Interruptions in the synthetic process and consequences of such interference can provide knowledge for the clinicians with powerful tool to control scar formation. Collagen synthesis by fibroblasts begins early in wound healing, by day 03 or 05 and continues for several weeks, depending on wound site.

COLLAGEN FIBERS

Functions of collagen include :

1. Support to the tissues.
2. Provides structural framework to other types of tissues.
3. Acts as a medium where blood vessels & nerves are passing.

4. Bring & keeps the wound edges together & provides tensile strength for holding together. This holding strength prevents the breakdown of tissue (organ) at the healed site.
5. Fill the gap caused by the tissue loss.

- Collagen is the most abundant [25% of total body protein]

  proteins of the connective tissue.

  Collagen is essentially a product of fibroblasts.

- **Collagen Deposition :** Collagen that gets deposited into the extra-cellular matrix of the healing wound has 4 successive phases of synthesis:
  1. Bio-synthesis of Tropo-collagen
  2. Fibril Formation
  3. Collagen Maturation
  4. Collagen Degradation.

Types of collagen.: 14 types of collagen can be discerned, of which the most well characterized are shown in following table.

Type of collagen	**CHAINS**				**Characteristi cs**	**Distribution**
I	α 1 (I),	α 2(I)			Bundles of banded fibers with high tensile strength	Skin (80%), Bone (90%), Tendons, Most other organs
II	α 1 (II)				Thin fibrils, Structural proteins	Cartilage (50%), Vitreous Humour
III	α 1 (III)				Thin fibrils, Pliable	Blood vessels, Uterus, Skin (10%)
IV	A 1	A 2	α 3	α 4,α 5, α 6 (IV)	Amorphous	All basement membranes
V	α 1 [V, α 2(V)]		α3(V)		Amorphous, Fine fibrils	2-5% of interstitial tissues, blood vessels, Interstitial tissues
VI	α 1 (VI)	α 2 (VI)	α3 (V)			
VII	α 1 (VII)				Anchoring Filament	Dermal-Epidermal Junction
VIII	α 1 (VIII)	A 2 (VIII)			Probably Amorphous	Endothelium-Descement's Membrane
IX	α 1 (IX)	α 2 (IX)	α3 (IX)		Probably Role in maturation of cartilage	Cartilage
X	α 1 (X)					
XI	α 1 (XI)	A 2 (XI), α				

Collagen Degradation:

•The static balance of collagen in a wound depends on both collagen production and also its degradation.

- It is achieved by the enzyme - Metalloproteinases.

GROUND SUBSTANCE IN HEALING WOUND:

- Connective tissue consists of cellular and non cellular component (matrix). Matrix is again composed of fibres and ground substance.
- **Definition:** This is non-fibrous part of the matrix in which cells and fibres are embedded.
- **Consistency:** Except in mineralized connective tissue, the ground substance is a viscous gel.

Constituents of Ground Substance

Water	**High proportion**
Mucopolysaccharides	It has been suggested that the fibroblasts, on the outer surface, have a layer of mucopolysaccharides whose charge and orientation determine the aggregation and orientation of tropocollagens.
Fibronectin	Fibronectin is a glycoprotein with high molecular weight There are two types of Fibronectin (a) Cell surface Fibronectin and (b) Plasma Fibronectin Functions: Fibronectin of connective tissue matrix acts as a glue between different matrix components and fibroblasts
Chondronectin	It is a specific adhesive between chondroblasts and type II
Mucoproteins	
Glycoproteins	
Lamenin	
Entactin	

WOUND CONTRACTION: [8]

- **Definition:** "Wound contraction is a process by which wound heals in a centripetal manner
- Wound contraction is one function of granulation tissue which is critical for repair.
- The events of wound healing from injury to fibroplasias, occurs in almost all wounds.

Certain events like wound contraction occurs characteristically in excision dermal wound and epithelization occurs in wounds of surface lining epithelium.

- In humans, the wound contraction is less because in most parts of the body the skin is somewhat firmly attached to subcutaneous tissue but it can occur in areas like back of neck and buttocks.
- **Timing of Wound contraction:**

Wound contraction starts from about 3rd or 4th day of wounding and continues up to 15th or 16th day and stops thereafter, irrespective of whether the wound is totally closed or not.

- **Rate of wound contraction:**

The rate of wound contraction is about 0.6-0.75 mm/day .

Wound contraction is not materially affected by size or shape of the wound but perhaps by the length of the wound perimeter.

Mechanism of wound contraction :

The mechanism of wound contraction is disputable and debatable. Many theories like Pull theory, Push theory / Picture Frame theory etc have been proposed but none of them appears to be satisfactory.

Wound contraction can be both beneficial or detrimental. Wound contraction can lead to distortion, disfigurement and impairment of function.

EPITHELIZATION

- Epithelization is a process of wound healing involving body surfaces.
- Unlike healing by fibroplasias where lost parenchymal cells are replaced by non- specific connective tissue, in epithelialization lost epithelial cells are replaced by epithelial cells only. It is an example of healing by regeneration.
- **Stages of epithelization**: The whole process of epithelization thus includes the following stages.
    1. Mobilization and loosening of basal cells from their dermal attachment.
    2. Migration or movement of cells to a position of cell deficit.
    3. Proliferation or replacement of cells to a position of cell deficit and

4. Differentiation or restoration of cellular function.

Epithelization which depends on several factors like:

1) Size of wound
2) Location of wound
3) Shape of wound
4) Impairment of blood supply
5) Pathological modification of wound.

- **Healing by epithelization occurs in:**
    1) Dermal wounds,
    2) Wounds of tracheobronchial surface,
    3) Surface wounds in gut, urinary bladder, uterus etc.

- Timing of Epithelization:

  First 24 Hrs of injury :-Changes in the epidermis leading to re-epithelization begin within 24 hours of the formation of a cutaneous wound.

- **MECHANISMS OF WOUND HEALING :**

Wound healing is a complex process of inflammation, proliferation, angiogenesis ,collagen maturation and wound contraction.

- **TYPES OF WOUND HEALING :**

Primary union – It is the healing of clean wounds with limited epithelial loss.ex-surgical incisions.

Secondary healing :

There is large component of tissue destruction, healing takes place by inflammation and wound contraction.

Growth factors:

Growth factors are secreted proteins from many tissues in the body exert varied effects on cell function. Growth factors stimulate or inhibit progression through the cell cycle that Control cell viability or death, or that act principally to regulate cell differentiation.

Their modes of action include Autocrine, Paracrine, juxtacrine, intracrine modes. Paracrine mode of action occurs when a growth factor that is secreted by one cell has an effect on adjacent cells. juxtacrine is similar as paracrine, although the growth factor is bound to the cell membrane or extra cellular matrix. Autocrine actions are mediated by a growth factor on its cell of origin after its secretion in to the extracellular environment. Intracrine actions occur inside the cell of origin. The effects of GF are mediated by activation of specific receptors. These receptors are transmembrane proteins.

Various Growth factors comparison[21,22]

Factor	Cell or Tissue of Origin	Selected Target Cells or Tissue	Selected Stimulatory (S) or Inhibitory (I) Actions	Clinical Trials
EGF	macrophages, monocytes	epithelium, endothelial cells	S: proliferation of keratinocytes, fibroblasts, and endothelial cells. S: keratinocyte migration.	venous ulcers
FGF	monocytes, macrophages, endothelial cells	endothelium, fibroblasts, keratinocytes	S: proliferation of endothelial cells, keratinocytes, and fibroblasts. S: chemotaxis, ECM	diabetic ulcers, venous ulcers, pressure ulcers
GMCSF	macrophages, fibroblasts, endothelial cells	hematopoietic, inflammatory cells, neutrophils, fibroblasts	S: chemotaxis of endothelial cells, inflammatory cells S: keratinocyte proliferation, activation of neutrophils	venous and arterial ulcers
HGH	pituitary gland	hepatocytes, bone, fibroblasts	S: IGF-1 production	venous ulcers
IL-1	lymphocytes, macrophages, keratinocytes	monocytes, neutrophils, fibroblasts, keratinocytes	S: monocytes, neutrophils S: macrophage chemotaxis	pressure ulcers
PDGF	platelets, macrophages, neutrophils, smooth muscle cells	fibroblasts, smooth muscle cells	S: proliferation of smooth muscle cells and fibroblasts S: chemotaxis S: ECM, contraction	diabetic ulcers, pressure ulcers
TGF-ß	platelets, bone, most cell types	fibroblasts, endothelial cells, keratinocytes, lymphocytes, monocytes	S: ECM, fibroblast activity S: chemotaxis I: proliferation of keratinocytes, endothelial cells	venous ulcers, pressure ulcers

* EGF = epidermal growth factor; FGF = fibroblast growth factor; GMCSF =granulocyte-macrophage colony-stimulating factor; HGH = human growth hormone; IL-1= interleukin-1; IGF-1 = insuling growth factor-1; PDGF = platelet-derived growth factor; TGF-ß = transforming growth factor-ß

PDGF[21]

It was discovered as a protein released from the alpha granules of platelets. It was purified from platelets as a highly basic 30- kilo delton dimeric protein. Purified PDGF was found to consist of two related chains, PDGF- A, PDGF-B, products of separate genes.

PDGF binds to receptors like PDGFR-α,PDGFR-β.They also are related in structure and sequence but are distinct gene products.

Both growth factors and their receptors are expressed in a wide variety of cell and tissue types. PDGF-BB has been prepared and purified for use in clinical studies of wound healing.

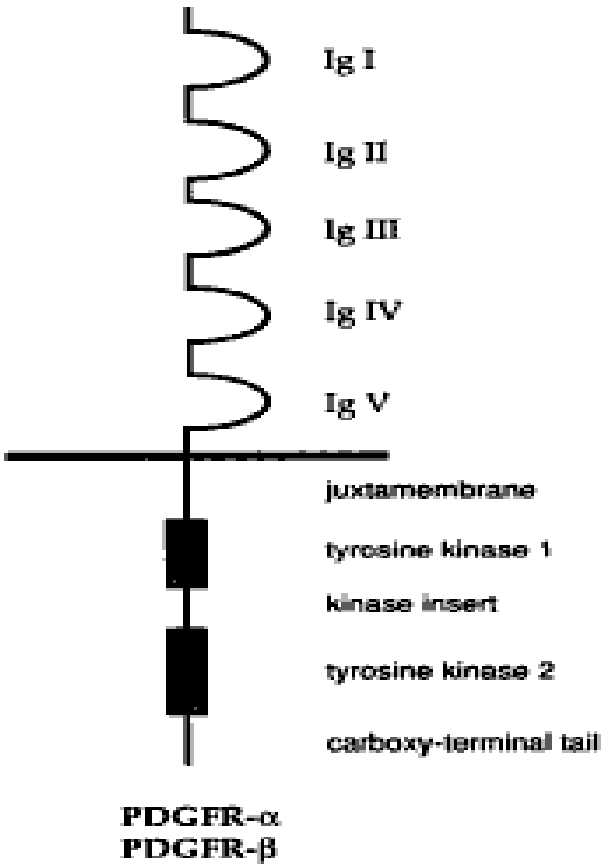
STRUCTURE OF PLATELET-DERIVED GROWTH FACTOR

Mature PDGF-A and -B chains are 109 amino acids in length and are 60% identical. All three combinations of growth factor dimers have been isolated from tissues: AA, AB, BB.In addition to platelet,PDGF has been isolated even from macrophage. Recently, two divergent members of the PDGF family were identified and termed PDGF-C and PDGF-D.[3]

PDGF RECEPTORS AND SIGNALING

The two PDGFRs are ligand-activated tyrosine protein kinases. The binding of PDGF to the extracellular region of the receptor induces receptor dimerization. Both homo- and heterodimets can form, depending on the ligand and the relative receptor abundance. PDGFR-β homodimers bind only PDGF BB and DD; PDGFR-α homodimers bind PDGF AA, AB, BB, and CC; whereas PDGFR-αβ heterodimers bind PDGF BB, AB, CC, and DD.

Structure of PDGF receptors[7]



BIOI.OGIC EFFECTS

PDGF action is essential for normal development. One of the major actions of PDGF in the adult is in wound healing. Tissue injury leads to the rapid

release of abundant PDGF A or B by degranulating platelets. Other short-term sources of growth factor include activated macrophages and endothelial cells. It is chemotactic for smooth muscle cells, fibroblasts, neutrophils, and monocytes and stimulates macrophage activation.

It is a potent mitogen for fibroblasts and smooth muscle cells and stimulates their proliferation in collaboration with other growth factors. PDGF induces expression of fibronectin, of collagenase, and of some types of remodelling that occurs during wound healing.

Various phase II and phase III studies showed effectively the efficacy, that is complete closure of the wound, and the reduction in the size of the wound. PDGF promote granulation tissue and stimulate cutaneous ulcer healing. It stimulates the proliferation of a variety of mesenchymal cells including fibroblasts.

DIABETES MELLITUS[23,24,25]

Definition :

Diabetes mellitus is a cluster of metabolic diseases causing chronic hyperglycemia from defects in insulin secretion,action or both. ·

Classification

TYPE I

Pathophysiology:

I A Autoimmune beta cell destruction Insulin Deficiency

I B Develop insulin deficiency by unknown mechanism causing destructive process of beta cells Lack immunologic markers

Type II

It is a heterogeneous group of disorders characterized by :-

- Impaired insulin secretion
- insulin resistance
- Increased blood glucose

Type 2 DM is usually preceded by impaired glucose tolerance.

Diagnosis

The National Diabetic Organisation have formulated a diagnostic criteria for DM-2 based on the following facts:

- RBS ≥200 mgs / dL Or ≥11.1 m mol / L with symptoms of DM (Polyuria, Polydipsia, Weight loss)
- FBS ≥126 mgs / dL or ≥ 7.0 mmol / L

- Strong co-relation b/w ↑ FPG & ↑ HbA1c concentration but currently not recommended for the diagnosis of DM.

Diagnosis of Diabetes Mellitus

Terms	Definition
Random Blood Glucose	Blood Glucose levels without regard to time since last meal
Fasting Blood Glucose	Blood Glucose levels when there is no caloric intake from past 8 Hrs

Neuropathy And Diabetes Mellitus

- Diabetic neuropathy prevalence increases by age,peak period is 60yrs.

  type1 & 2 DM.
- May manifest as Poly/Mono neuropathy or Autonomic Neuropathy

All nerve fibres are affected,since symptoms of diabetic neuropathy are similar to other types of neuropathy,various causes should be excluded.

Poly-neuropathy / Mono-neuropathy[26,27,28] **:**

- The most common form of diabetic neuropathy is distal equivocal polyneuropathy.
- It presents as:
  1. sensory loss - common presentation
  2. Hyperesthesia
  3. Paresthesia
  4. Dysesthesia

- Symptoms includes a sensation of following, which begins in the feet & spreads proximally

    1. Numbness
    2. Tingling
    3. Sharpness
    4. Burning

- Physical examination reveals loss of sensation, ankle reflex loss and deranged position sense.

treatment of diabetic neuropathy :

- Adequate glycaemic control
- Vitamin B12 and folate supplementation
- Analgesics

Lower Extremity Complications

One of the major causes of morbidity in DM patients is foot ulcer and chronic infection.

- The causative factors are microvascular disease, deranged biomechanics of the foot, neuropathy and wound infection.

Neuropathy :

It is the major cause of foot ulcers in diabetic patients with a prevalence of 80%.

Motor and sensory neuropathy :

Structural deformity of foot like claw toe,charcot joint occurs due to motor and sensory neuropathy.

Peripheral sensory neuropathy :

The normal foot biomechanics are lost resulting in trauma to the foot repeatedly. Often the patient is unaware of the trivial foot injuries.

Autonomic neuropathy :

Causes dryness of the foot resulting in fissures and ulcers.

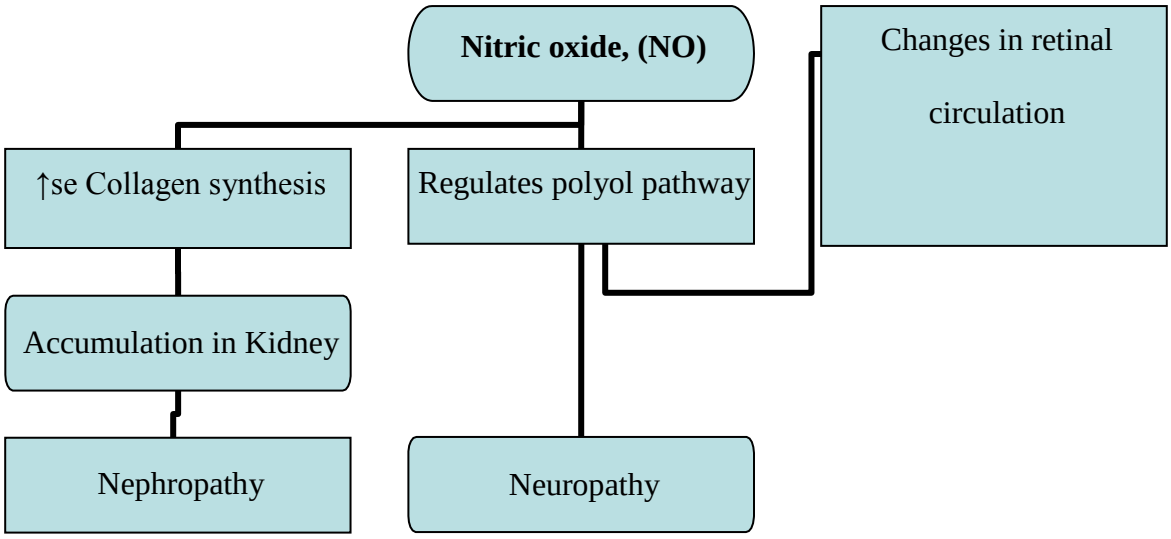
VASCULAR CHANGES IN DIABETES[29]

Atherosclerosis: It's a process of chronic accumulation of intraluminal plaque.

DM causes rapid development of atherosclerosis, Eventually atherosclerosis causes thrombosis of the vessel resulting in varied symptoms or dislodges as an emboli.

II. Endothelium:

a. Nitric oxide, (NO): (EDRF-Endothelium derived relaxing factor)



b. Prostacyclin (PGI_2)

Potent vasodilator also inhibits,platelet adhesion & aggression.Decreased in Diabetics & arterial wall of patients with old MI

c. Thromboxane A_2($TX\text{-}A_2$):>>>>Vasoconstrictor- Conteracts effect of N.O

decreased levels found in DM, HTN & hyperlipidemia.

d. Endothelin::>>>>Vasoconstrictor

decreased levels found in DM around 3.5 times.

Pathogenesis of diabetic ulcers.

Diabetes

Mellitus

↓

Neuropathy & limited joint

mobility

↓

Atrophy of intrinsic muscles & clawing

Pressure transfer from heel & toes to metatarsal heads

↓

No redistribution of

pressure

↓

Ulceration

Predisposing factors for ulceration: [30]

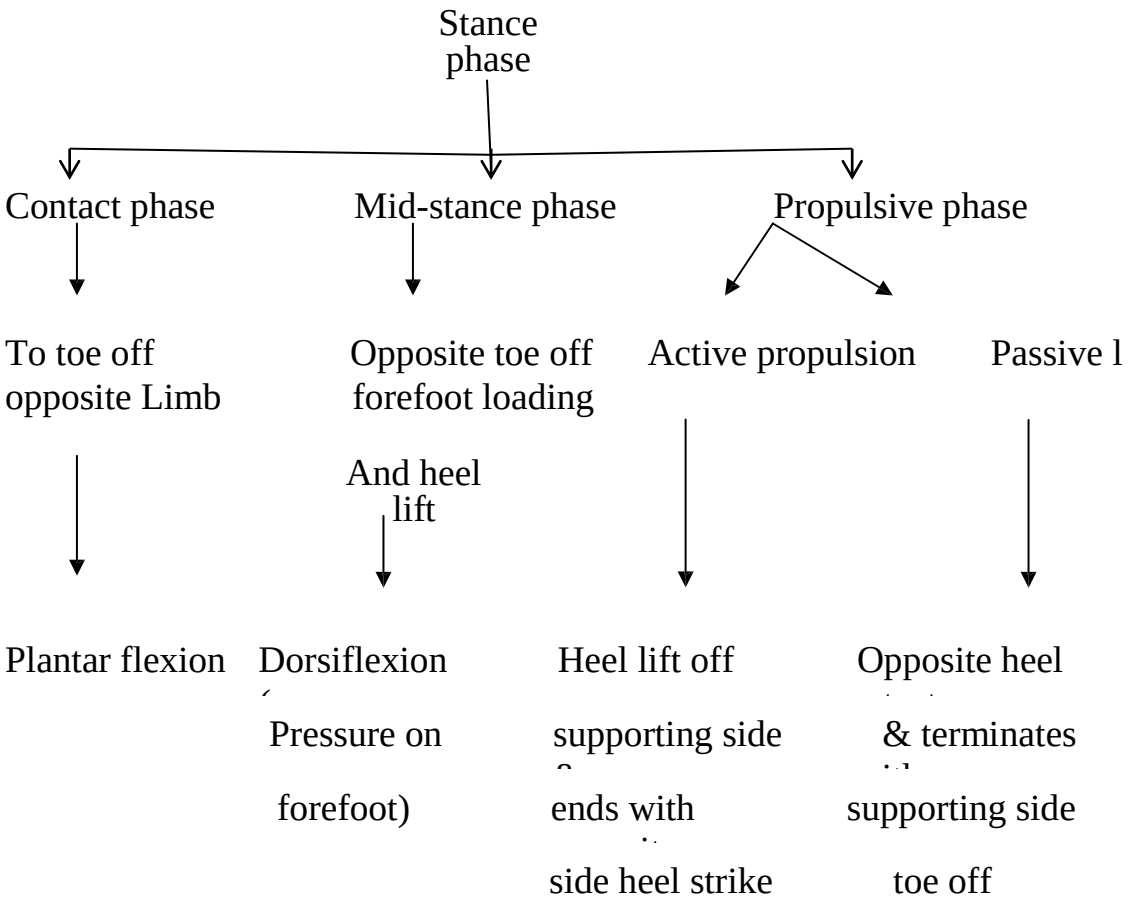
1) Limited joint mobility.

2) Peripheral neuropathy.

3) High plantar pressure.

4) Vascular diseases.

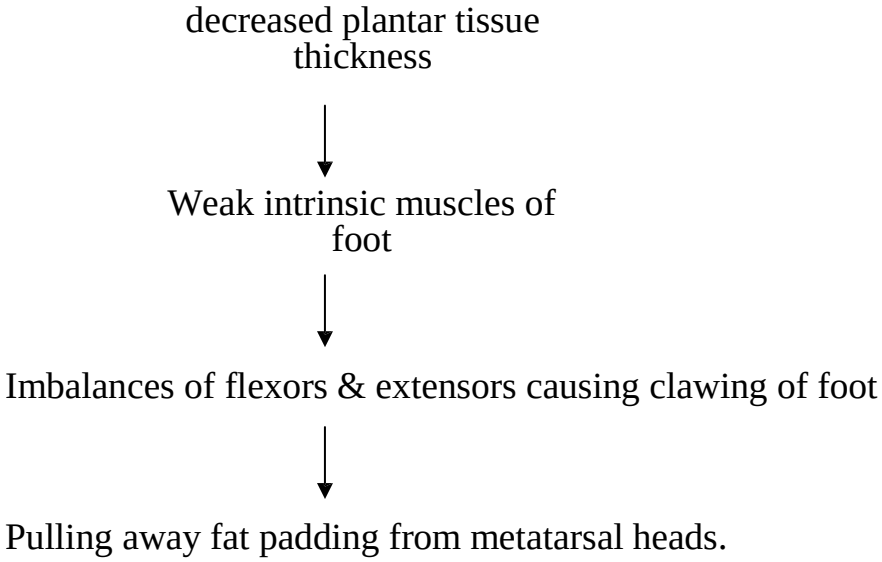
1. **Biomechanics of diabetic foot**



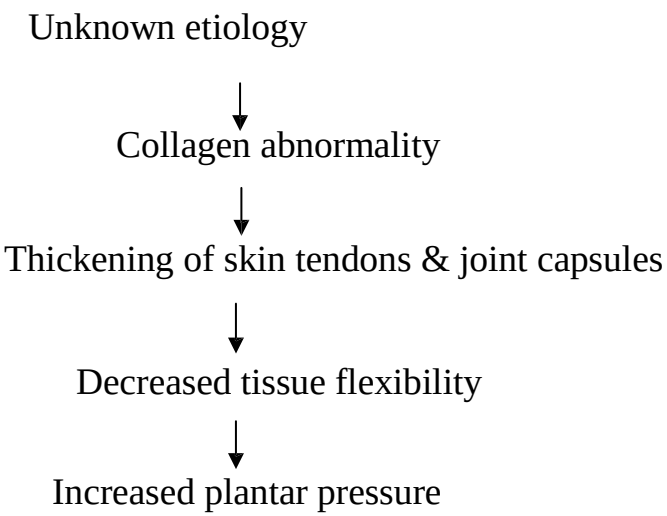
Changes in foot caused by diabetes[31]

1. Peripheral neuropathy
    A. Dryness of skin
    B. Callus formation

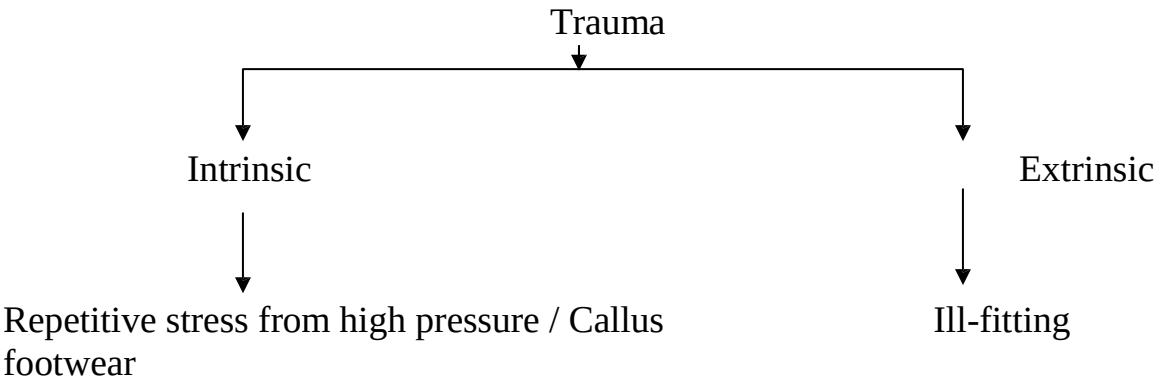
2. High pressure at bony prominences



3 Limited joint mobility



4 Trauma



CLASSIFICATION OF DIABETIC FOOT ULCERS. [32]

There are various models which classify Diabetic ulcer. The Wagner classification is one such classification which is commonly used It classifies wounds by the extent of ulceration.

WAGNER CLASSIFICATION:

Grade 1: Superficial Diabetic Ulcer

Grade 2: Diabetic Ulcer Involving the fascia, ligament, joint capsule or tendon

Grade 3: Deep ulcer with abscess or osteomyelitis

Grade 4: Gangrene of the forefoot

Grade 5: Extensive gangrene of foot.

The International Working Group on Diabetic Foot has proposed the PEDIS classification, which grades the wound in accordance to

- Extent (area)
- Sensation
- Depth
- Infection
- Tissue Perfusion

WOUND DRESSING IN DIABETIC FOOT

The management of wound and wound dressing is an important aspect of diabetic foot management. Proper dressing with cost effective dressing material, done with scientifically correct method can help in salvaging diabetic foot. The various functions of the dressings are:

- Isolation of the wound from external environment.
- Limit/reduce tissue oedema.
- Reduce pain.
- Improve gas exchange between tissues and blood.
- Limit inflammation.
- Absorb exudate.
- Should not promote bacterial growth.
- Prevent desiccation and contamination.

All the dressings can be classified as primary or secondary. Primary is the one, which covers the wound and is in direct approximity to the wound.While the Secondary dressing which holds the primary dressing in place is the secondary dressing. It has function of compression, occlusion and additional protection.

VARIOUS TYPES OF DRESSINGS

A wide variety of dressing solutions are available for dressing of infected diabetic foot ulcers.

a. **Eusol**: contains bleaching powder and boric acid. Acts by chemical desloughing of the wound.
b. **Collagenase dressing**: contains collagenase enzyme which helps in the break down of devitalized tissues.
c. **PDGF gel**: contains the rh-growth factor Causing angiogenesis and formation of healthy granulation tissue.

d. **Comupimet** ointment: contains collagen crystals with Mupirocin and Metronidazole. Acts by enhancement of granulation tissue along with antibacterial action

e. **Aquacell**: contains silver ions, which has anti-microbial action. Helps in cleansing the wound.

f. **Biological dressings**:

   i. APLIGRAFT- Bioengineered skin

   ii. DERMA GRAFT- Human dermis

MATERIALS AND METHODS

Study design: Prospective Randomized controlled trial.

Source of Data : Patients with diabetic foot ulcers admitted in surgery wards at PSG Hospitals over a period from august 2011 to august 2012

Sample Size :

50 patients

25-patients -in the study group

25-patients -in the control group

Inclusion criteria

1) Age between 20 and 80 yrs.

2) Diabetic ulcers located below the ankle.

3) Diabetic ulcers of the leg-Grade 1 and 2(Wagner classification)

4) Size of ulcer less than 10x10 cm[length X breath]

Exclusion criteria:

1) Poor sugar control(HbA1c >12%)

2)Patients with severe Anaemia(<7gm/dl)

3)Diabetic ulcers grade 3,4 and 5(Wagner classification)

.

Method

The present study was carried out at PSG Hospitals, Coimbatore, for a period of one year, where 50 patients with diabetic foot ulcers were included in the present study. Using a pretested and predesigned proforma the study population was randomized into either study group or control group using an open label randomization technique. Out of 50, patients, 25 took treatment in the form of conventional normal saline dressings and 25 took treatment with rh-PDGF dressing once a day.Glycaemic control and adequate control of infection was maintained in both the groups. If culture grows organism, both control and study group cases would be treated with antibiotics as per culture sensitivity report.X-ray foot was taken for all patients and bony involvement was excluded.The initial area measurement was taken on day 01 and final area measurement on day 15 was taken on transparent sheet. Plannimetry was used to measure the outcome that is the target ulcer area using a transparent graph sheet. Results were calculated by using student 't' test.

DRESSING TECHNIQUE

For saline dressing.

The ulcer was cleaned with normal saline and saline soaked gauze piece was kept over the ulcer which was covered with pad and roller bandage.

For rh-PDGF dressing

The infected ulcer was cleaned with Normal Saline. Commercially available rh-PDGF-BB gel (0.01%) was applied on the gauze piece and put on the ulcer. It was then covered with pad and roller bandage.

The dressings were changed daily morning in both control and study group for 15 days and appearance of healthy granulation tissue is observed. The initial area and final area of the size of the ulcer are measured on 15^{th} day by planimetry[33,34,35] using a transparent graph sheet and subjected to statistical analysis.

The following formula was used to calculate % reduction in area of wound after 15 days period in both cases and control groups.

Rate of contraction of wound after 15 days of treatment =

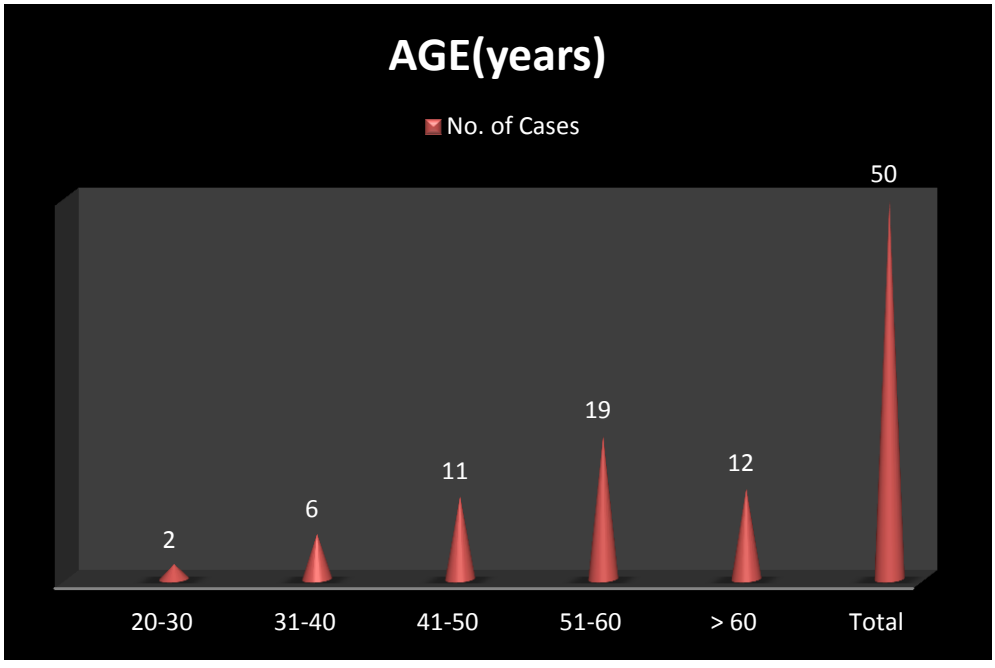
$$\frac{\text{(Initial area – Final Area)}}{\text{Initial are}} \text{ X } 100$$

OBSERVATIONS AND RESULTS

Age Distribution

Age (Years)	No. of Cases	Percentage
20-30	02	4%
31-40	06	12%
41-50	11	22%
51-60	19	38%
> 60	12	24%
Total	50	100

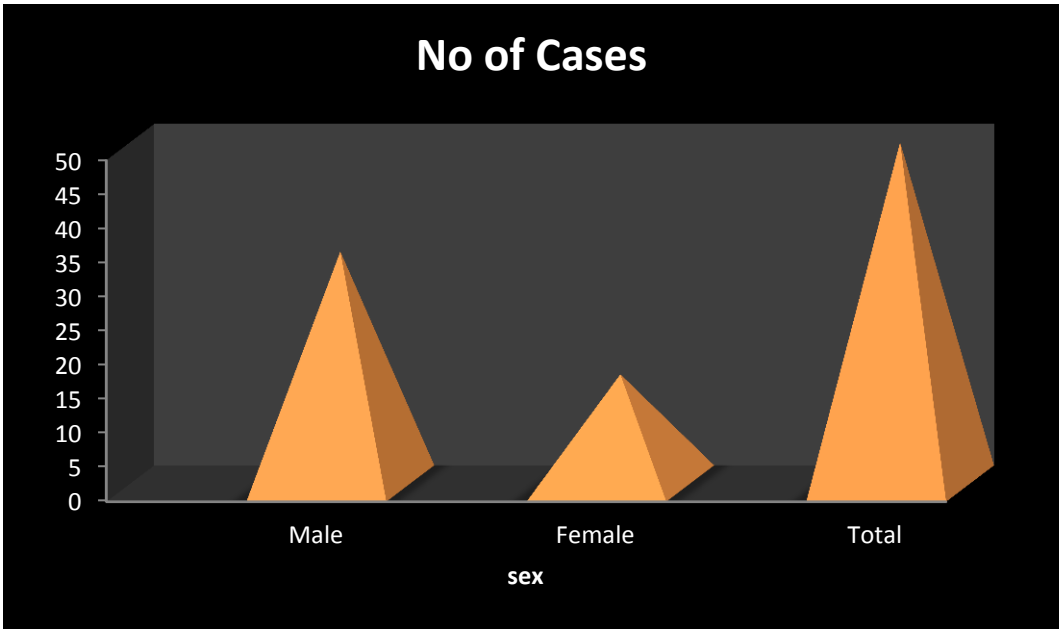
In our study it was observed that Diabetic foot was commonest in the age group between 51-60 yrs of age.



Sex Distribution

Sex	No of Cases	Percentage
Male	34	68.00%
Female	16	32.00%
Total	50	100.

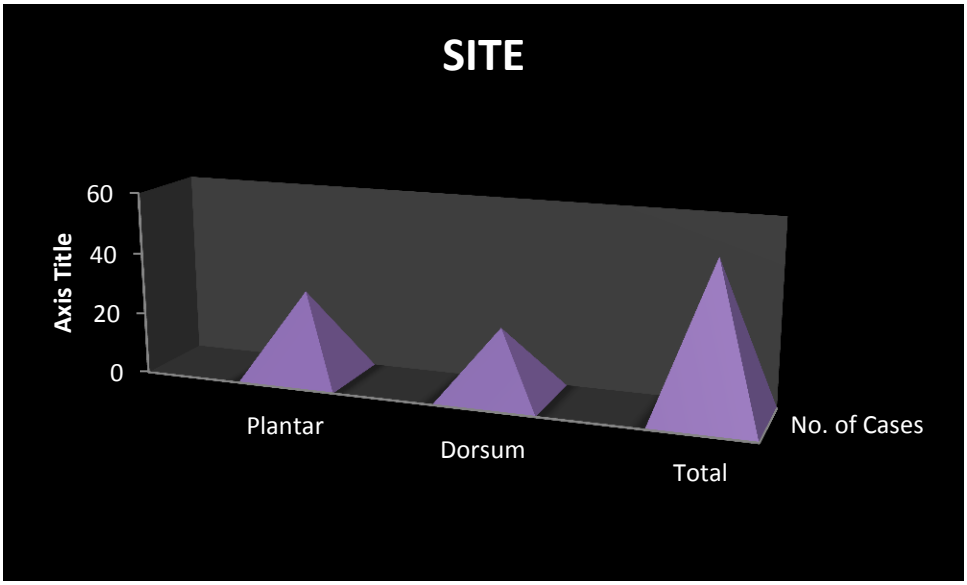
In our study in was observed that Diabetic foot was more common in the males (68.00%) as compared to females (32.00%).



Site of ulcer in the study

Site	No. of Cases	Percentage
Plantar	28	56.00%
Dorsum	22	44.00%
Total	50	100%

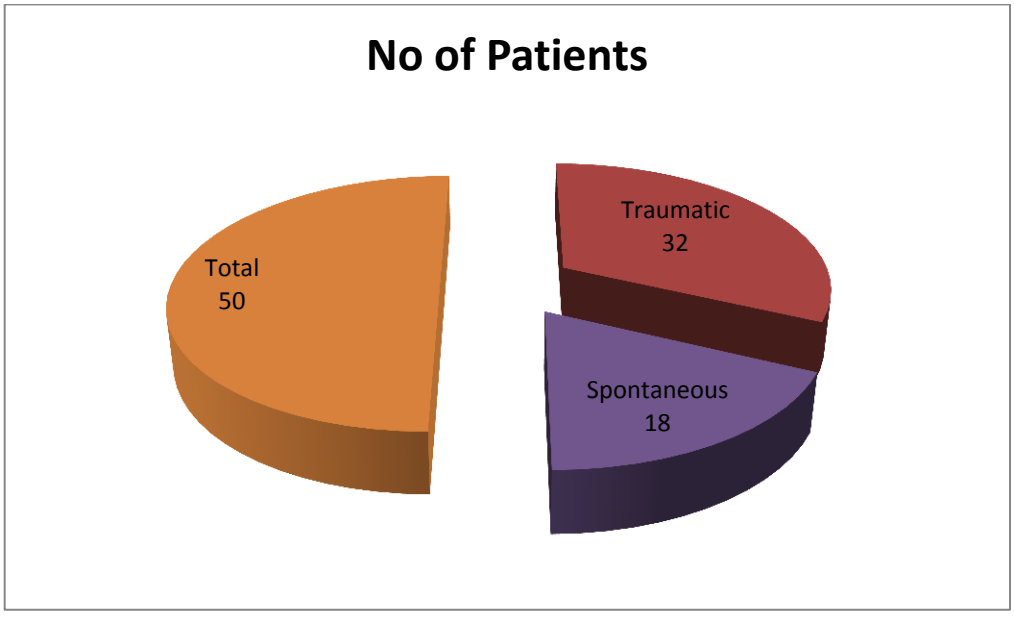
In our study it was observed that diabetic foot more commonly occurs on the plantar aspect (56%) of the foot as compared to the dorsal aspect (44%).



Onset of Diabetic Foot Ulcers

Type of Onset	No of Patients	Percentage
Traumatic	32	64.00%
Spontaneous	18	36.00%
Total	50	100

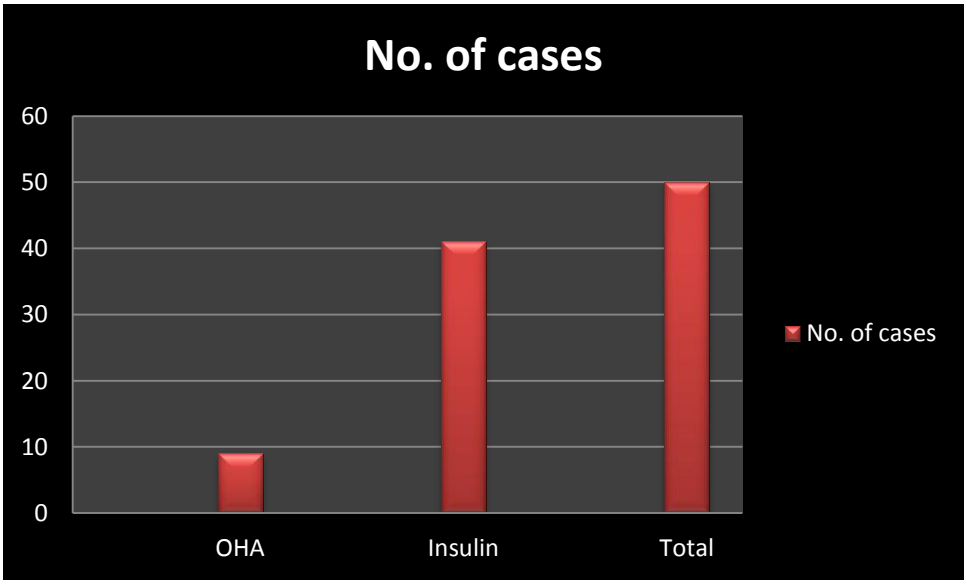
Trauma is the most common cause of diabetic foot ulcer (64%) while only 36%were spontaneous in origin.



Anti Diabetic Agents

Anti Diabetic	No. of cases	Percentage
OHA	9	18.00%
Insulin	41	82.00%
Total	50	100%

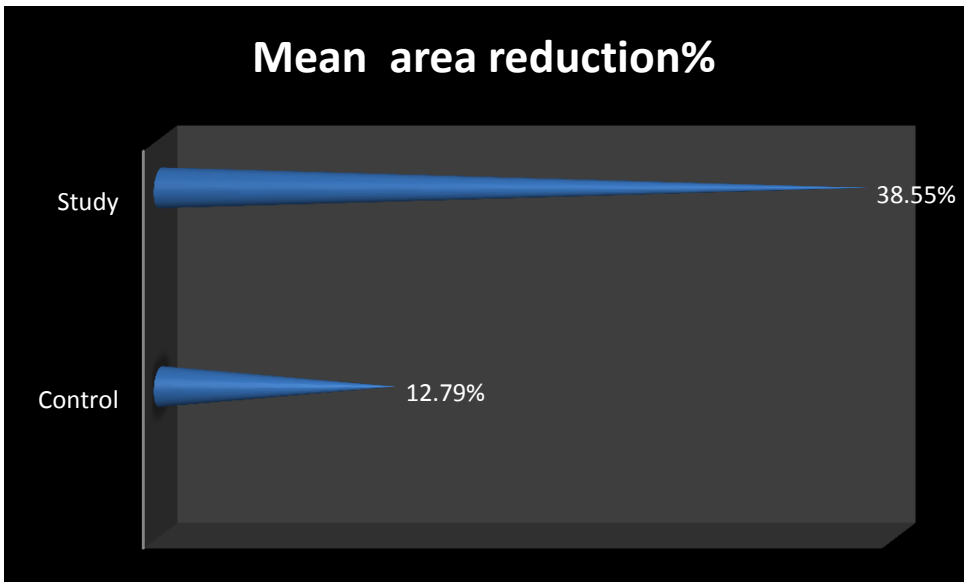
In our study most of the participants were taking Insulin for glycaemic control.



Wound Contraction

Group	Mean area reduction%	S.D.	Median	P Value
Control	12.79%	2.55	11.81	
Study	38.55%	2.52	37.58	P<0.001

In our study it was observed that Mean % of area reduction was higher in study group (38.55%) as compared to the controls (12.79%).



Diabetic foot ulcers in the study group had better mean % of wound contraction of 38.55% as compared to the control group which had mean % of wound contraction of 12.79 %, the difference in the mean 25.76% of area reduction of the two groups where studied using unpaired student t test was found to be significant ($p<0.001$).

Diabetic Foot ulcers(study group) treated with PDGF:

BEFORE TREATMENT

AFTER TREATMENT

Diabetic Foot ulcers(study group) treated with PDGF:

BEFORE TREATMENT

AFTER TREATMENT

DISCUSSION

Diabetic foot ulcers are chronic wounds, with prolonged inflammation phase and shows cessation of epidermal growth. Invariably the diabetic foot ulcers are resistant to heal because of multidrug resistant organism growth and microvascular complications.

The present study was conducted at PSG hospital,coimbatore to study the effect of use of PDGF in diabetic foot ulcers.In the present study it was seen that the incidence of diabetic foot ulcers were more in males (68%) as compared to females (32%). The second national data source, NHDS documented more males suffering from diabetic foot ulcer than females.

Diabetic foot ulcers are most commonly seen in 5^{th} decade (38%), the next common being in the sixth decade .While only 22% of the patients were in the fourth decade.Older the patient more the prevalence of having diabetic foot ulcer. In this study patients with vascular complications such as pulse less limb and the patients with osteomyelitis were excluded.

In this study, 64% of the ulcers were traumatic in origin, trauma being the triggering factor secondary to neuropathy. 36% were spontaneous in origin secondary to blister rupture or unnoticed trivial trauma.

More than half (56%) of the patients had ulcer on the plantar surface of the forefoot and the remaining (44%) had on the dorsum of foot. Study conducted by Edmonds et al. (Edmonds)[36] showed more foot ulcers were on plantar and fore foot areas. Most of the diabetic foot ulcers are invariably due to poor foot care and due to gait abnormalities.

Most of the patients (82%) were on insulin for control of sugar whereas only 18 % were on Oral Hypoglycaemic Agents.In our study it was observed that participants receiving rh-PDGF dressing had better wound contraction of 38.55.As compared to the group receiving only conventional dressing (normal saline dressing) in whom the mean wound contraction was 12.79% these were found to be statistically significant on unpaired Student t test ($p<0.001$) suggesting that rh-PDGF dressing enhances wound healing in diabetic wounds.

We have found 12.79% (S.D; 2.55: Median; 11.81) contraction of wounds in the control groups as compared to 38.55% (S.D; 2.52, Median; 37.58) contraction of wounds in study group. Therefore, study groups are having 25.76 % more wound contraction as compared to control group. On applying unpaired student t test $p<0.001$ which is Statistically significant. From our study, we can say that rh-PDGF dressing therapy facilitates wound healing in patients suffering from diabetes mellitus.

CONCLUSION

The wounds in the study group treated with rh-PDGF dressing contracted more than the wounds in the control group (38.55% Vs. 12.79%; P = < 0.001- Statistically Significant) which indicates rh-PDGF dressing is an effective modality to facilitate wound contraction in patients suffering from diabetes. Rh-PDGF dressing is found to be more effective, safe promoter of wound healing and can be used as an adjunct to saline dressings for healing of diabetic wounds.

BIBLIOGRAPHY

1) The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20: 1183–1197, 1997

2) Reiber GE, Ledous WE. Epidemiology of diabetic foot ulcers and amputations: Evidence for prevention. In: Williams R, Herman W, Kinmonth A-L, et al., eds. The evidence base for diabetes care. London: John Wiley & Sons; 2002. p. 641-665.

3) Heldin CH. "Structural and functional studies on platelet-derived growth factor". EMBO J. 11 (12): 4251–4259. (1992)

4)Sipos P, Gyõry H, Hagymási K, Ondrejka P, Blázovics A (February 2004). "Special wound healing methods used in ancient Egypt and the mythological background". World J Surg 28 (2): 211–6

5)Ovington LG. "The evolution of wound management: ancient origins and advances of the past 20 years". Home Healthc Nurse 20 (10): 652–6. (October 2002).

6)Forrest RD. Early history of wound treatment. J R Soc Med 1982; 75(3): 198-205.

7) Stein JM, Levenson SM. Effect of the inflammatory reaction on subsequent wound healing in rats. Acta Chir Scand 1964;127:446, 125.

8)Bennett, N. T. and Schultz, G. S. Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am. J. Surg. 165, 728-737. (1993).

9) Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, et al. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 2008;16(1):2–10.

10)Lei KJ, Alitalo K, Maglione D, Guerriero V, Viglietto G, Ferraro MG, Aprelikova O, Chou JY, Persico MG, Del Vecchio S (1993). "Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14". Oncogene 8 (4): 925–931.

11)SANDBLOM P, MUREN A. Differences between the rate of healing of wounds inflicted with short time interval. I. Cutaneous incisions. Ann Surg. 1954 Oct;140(4):449–458.

12)Khurana R, Simons M (April 2003). "Insights from angiogenesis trials using fibroblast growth factor for advanced arteriosclerotic disease". Trends in Cardiovascular Medicine 13 (3): 116–22.

13)Thurston G (October 2003). "Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymph angiogenesis". Cell and Tissue Research 314 (1): 61–8. doi:10.1007/s00441-003-0749-6

14)David M. Mosser and Justin P. Edwards (December 2008). "Exploring the full spectrum of macrophage activation". Nature Reviews Immunology 8 (12): 958–969.

15)St Pierre BA, JG Tidball (1994). "Differential response of macrophage subpopulations to soleus muscle reloading following rat hindlimb suspension". Journal of Applied Physiology 77

16)Lucas AD, Greaves DR (November 2001). "Atherosclerosis: role of chemokines and macrophages". Expert Rev Mol Med 3 (25): 1–18.

17)Olsen SK, Garbi M. et al. (2003). "Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs". J. Biol. Chem. 278 (36): 34226–36

18)Gospodarowicz D (1974). "Localization of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth". Nature 249 (453): 123–7.

19)Murzin AG, Lesk AM, Chothia C (January 1992). "beta-Trefoil fold. Patterns of structure and sequence in the Kunitz inhibitors interleukins-1 beta and 1 alpha and fibroblast growth factors".

20)Traub, W.; Yonath, A. & Segal, D. M. (1969). "On the molecular structure of collagen". *Nature* 221 (5184): 914–917. Bibcode 1969Natur.221..914T

21)Shulman T, Sauer FG, Jackman RM, Chang CN, Landolfi NF (July 1997). "An antibody reactive with domain 4 of the platelet-derived growth factor beta receptor allows BB binding while inhibiting proliferation by impairing receptor dimerization". J. Biol. Chem. 272 (28): 17400–4.

22)Joukov V, Pajusola K, Kaipainen A, Saksela O, Alitalo K, Olofsson B, von Euler G, Orpana A, Pettersson RF, Eriksson U (1996). "Vascular endothelial growth factor B, a novel growth factor for endothelial cells"Acad. Sci. U.S.A. 93 (6): 2567–2581

23)Williams textbook of endocrinology (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435.

24)Lawrence JM, Contreras R, Chen W, Sacks DA (May 2008). "Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005". Diabetes Care 31 (5): 899–904.

25)Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, Byrd-Holt DD (April 1998). "Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988–1994". Diabetes Care 21 (4): 518–24

26)Stewart WF, Ricci JA, Chee E, Hirsch AG, Brandenburg NA (June 2007). "Lost productive time and costs due to diabetes and diabetic neuropathic pain in the US workforce". J. Occup. Environ. Med. 49 (6): 672–9.

27)Bastías A MJ, Toro C L, Olmos C P (December 2006). "[Intensified insulin therapy plus antineuritic medication is more effective than antineuritics alone in painful diabetic neuropathy]". Rev Med Chil 134 (12): 1507–15.

28)Typpo, Omaha (2010-12-26). "Importance of Physical Activity in Neuropathy". Demand Media Inc. Retrieved 2011-05-03.

29)Rader A, Barry T (Nov/Dec 2006). "A proposed mechanism for pain relief following NIR or MIRE therapy". Diabetic Microvascular Complications Today: 27–8.

30)Wong MC, Chung JW, Wong TK (2007). "Effects of treatments for symptoms of painful diabetic neuropathy: systematic review". BMJ 335 (7610): 87

31)Moreo, Kathleen (2005). "Understanding and overcoming the challenges of effective case management for patients with chronic wounds". The Case Manager 16 (2): 62–3, 67.

32) Brem Harold, Tomic-Canic Marjana (2007). "Cellular and Molecular basis of wound healing in diabetes". *JCI* 117 (5): 1219–1222.

33)Margolis DJGross EAWood CRLazarus GS Planimetric rate of healing in venous ulcers of the leg treated with pressure bandage and hydrocolloid dressing. J Am Acad Dermatol. 1993;28418- 421

34)Tallman PMuscare ECarson PEaglstein WHFalanga V Initial rate of healing predicts complete healing of venous ulcers. Arch Dermatol. 1997;1331231- 1234

35)Wysocki AB Wound measurement. Int J Dermatol. 1996;3582- 91.

36)Edmonds M.The diabetic foot,2003.Diabetes Metab Res Rev.2004;20(suppl 1):S9-S12.

PROFORMA

I) PATIENT IDENTIFICATION DATA:

NAME IP/OPD NO

AGE SEX

DOA: OCCUPATION:

DOD:

ADDRESS

II) CHIEF COMPLAINTS:

MEDICAL HISTORY:

Peripheral Neuropathy:

Nephropathy

Retinopathy

DIABETIC STATUS:

TYPE:

DURATION:

MEDICATION: Oral Hypoglycemic Insulin

( ) ( )

COMPLICATION Neuropathy ( )

Vasculopathy ( )

ULCER DETAIL :

1. Mode of onset

Traumatic ( )

Spontaneous ( )

Pressure ( )

Others ( )

2. Duration

3. Progress

WOUND OBSERVATION:

1. Site
2. Size
3. Shape
4. Edge
5. Margin
6. Floor
7. Base
8. Discharge
9. Surrounding Skin

NERUROLOGICAL EXAMINATION :

VASCULAR EXAMINATION

	Right	Left
Popliteal artery		
Ant . Tibial artery		
Post tibial artery		
Dorsalis pedis artery		

INVESTIGATIONS.

CBC

FBS

Hba_1c

Sr. Creatinine

UKB

Urine : Routine

Microscopy

X-ray Foot

AP View

Lat. View

Wound C/s

WOUND AREA MEASUREMENT ON D_1 in cm^2

Type of Dressing – saline dressing ( )

- rh-PDGF dressing ( )

MASTER CHART-STUDY GROUP

Sl. no	Ip.no	Age & sex	0nset	Site	Anti DM Rx	Initial Area in mm^2	Final Area in mm^2	IA-FA= CA	%Area Reduct- ion
1	I11019046	50/M	T	P	I	1321.8738219.003	219.643	1102.234	38.65
2	I11019267	40/M	T	D	O	3181.937	2060.9639	1120.9740	34.23
3	I11020569	46/F	S	P	I	3563.038	2110.0311	1453.0060	41.78
4	I11021373	46/F	T	D	O	1783.993	1090.5549	693.4390	37.87
5	I11019634	60/M	S	D	I	2735.654	1656.4385	1097.2150	38.45
6	I11019336	55/M	T	P	I	1563.836	942.3675	621.4690	38.74
7	I11017586	50/F	T	D	O	2536.974	1625.6929	911.2811	34.92
8	I11018576	51/M	T	P	I	1535.736	890.2661	645.4699	41.03
9	I11019934	70/F	T	P	O	1432.388	881.6348	550.7532	37.45
10	I11016797	56/M	S	D	I	2536.84	1592.3745	944.4700	36.23
11	I11019697	64/M	T	P	O	1535.736	890.2661	645.4699	41.03
12	I11016558	56/M	T	D	I	1432.388	881.6348	550.7532	37.45
13	I11018576	50/M	S	D	O	3231.937	2010.9112	1221.0260	36.78
14	I11017278	70/F	T	P	O	2435.836	1340.197	1095.6390	43.98
15	I11020382	54/M	T	P	I	3216.073	1729.2825	1486.7900	45.23
16	I11017802	50/F	T	D	I	2433.947	1498.0944	935.8520	37.45
17	I11017472	60/M	S	P	I	2433.764	1517.9386	915.8250	36.63
18	I11019021	75/M	S	D	I	3546.836	2282.7436	1264.0920	34.64
19	I11017700	65/F	T	D	I	2435.635	1393.6703	1041.9600	41.78
20	I11021390	29 /M	T	P	I	2194.937	1241.0174	953.9205	42.46
21	I11017638	30/M	S	P	I	3425.846	1971.5744	1454.2716	40.45
22	I11019235	74/M	S	P	I	3487.833	2108.7438	1379.0900	37.54
23	I11020762	51/M	S	D	O	2369.837	1378.2972	991.5398	40.84
24	I11016307	68/F	T	D	I	3598.937	2273.4485	1325.4885	37.83
25	I11020795	51/M	T	P	I	3563.038	2110.0311	1453.0060	39.78

MASTER CHART-CONTROL GROUP

Sl No.	I P No.	Age/ Sex	Onset	Site	Anti-DM Rx	Initial Area mm^2	Final Area mm^2	IA - FA = CA	% Area Reduct- ion
1	I11021660	42/M	T	P	O	3546.882	3186.164	360.718	11.17%
2	I11020851	75/M	T	P	I	3134.45	2845.141	289.309	10.23%
3	I11023046	55/M	S	P	I	2673.993	2360.066	313.927	12.74%
4	I11034652	38/F	S	D	I	3547.273	3078.678	468.595	14.21%
5	I11021390	69/M	T	P	I	2534.45	2271.881	262.569	11.36%
6	I11023570	38/M	T	D	I	1344.994	1153.013	191.981	15.27%
7	I11032995	40/M	T	P	O	3564.98	3217.038	347.942	10.76%
8	I11021801	55/M	T	P	I	3365.45	2875.441	490.009	15.56%
9	I11033103	65/M	T	P	O	2673.83	2402.703	271.127	11.14%
10	I11020569	67/F	T	D	I	2893.003	2619.324	273.679	10.46%
11	I11032726	68/M	S	D	I	2663.748	2228.491	435.748	17.34%
12	I11021373	69/F	T	P	O	2513.734	2278.699	235.035	10.35%
13	I11034765	38/F	T	P	I	2436.748	2163.101	273.647	12.23%
14	I11034156	60/M	T	P	I	3226.56	2895.514	331.046	11.26%
15	I11033177	70/F	S	P	I	3456.643	3123.768	332.875	10.63%
16	I11021623	46/M	T	D	I	2263.744	1934.142	329.602	16.56%
17	I11034271	52/M	T	P	O	2673.83	2402.703	271.127	11.14%
18	I11034998	45/F	T	P	I	3428.923	2961.903	467.023	14.62%
19	I11034925	41/F	S	D	I	2283.485	1887.528	395.957	18.34%
20	I11033920	60/M	T	P	I	3546.875	3147.496	399.406	12.26%
21	I11033995	45/M	T	D	I	3317.992	2941.731	376.261	12.34%
22	I11033621	67/F	T	P	I	1945.644	1766.061	179.583	10.23%
23	I11033920	51/M	T	D	I	1925.773	1662.905	262.868	14.65%
24	I11021795	40/M	S	D	O	3187.093	2780.736	406.357	13.75%
25	I11022775	66/M	T	D	O	1672.934	1391.379	281.545	18.83%